Human induced pluripotent stem cell line with genetically encoded fluorescent voltage indicator generated via CRISPR for action potential assessment post-cardiogenesis.

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Public Summary:
Genetically encoded fluorescent voltage indicators, such as ArcLight, have been used to faithfully report action potentials (APs) in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). However, the ArcLight expression, in all cases, relied on a high number of lentiviral vector-mediated random genome integrations (8-12 copy/cell), raising concerns such as gene disruption and alteration of global and local gene expression, as well as loss or silencing of reporter genes after differentiation. Here, we report the use of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 nuclease technique to develop a hiPSC line stably expressing ArcLight from the AAVS1 safe harbor locus. The hiPSC line retained proliferative ability with a growth rate similar to its parental strain. Optical recording with conventional epifluorescence microscopy allowed the detection of APs as early as 21 days postdifferentiation, and could be repeatedly monitored for at least 5 months. Moreover, quantification and analysis of the APs of ArcLight-CMs identified two distinctive subtypes: a group with high frequency of spontaneous APs of small amplitudes that were pacemaker-like CMs and a group with low frequency of automaticity and large amplitudes that resembled the working CMs. Compared with FluoVolt voltage-sensitive dye, although dimmer, the ArcLight reporter exhibited better optical performance in terms of phototoxicity and photostability with comparable sensitivities and signal-to-noise ratios. The hiPSC line with targeted ArcLight engineering design represents a useful tool for studying cardiac development or hiPSC-derived cardiac disease models and drug testing.

Scientific Abstract:
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